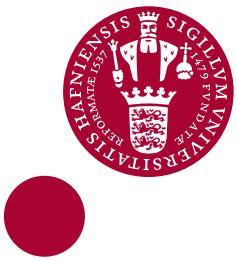

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Neonatal vitamin D status from archived dried blood spots and future risk of fractures in childhood: results from the D-tect study, a population-based case-cohort study

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ABSTRACT

Background: Whether antenatal and neonatal vitamin D status have clinical relevance in fracture prevention has not been examined extensively, although observational studies indicate that fetal life may be a sensitive period in relation to bone growth and mineralization during childhood.

Objective: We examined whether 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations in stored neonatal dried blood spot (DBS) samples are associated with pediatric fracture risk. We hypothesized that in particular, low neonatal vitamin D status may be a risk factor for fracture incidence among children.

Design: In a register-based case-cohort study design, the case group was composed of 1039 individuals who were randomly selected from a total of 82,154 individuals who were born during 1989–1999 and admitted to a Danish hospital with a fracture of the forearm, wrist, scaphoid bone, clavicle, or ankle at age 6–13 y. The subcohort was composed of 1600 individuals randomly selected from all Danish children born during 1989–1999. The neonatal 25(OH)D_3 concentrations in DBS samples were assessed by using highly sensitive chromatography-tandem mass spectrometry.

Results: The mean ± SD 25(OH)D_3 concentration for all subjects was 27.7 ± 18.9 nmol/L [median (IQR): 23.5 nmol/L (13.3, 37.3 nmol/L)] and showed significant monthly variation ($P < 0.0001$) with the highest values in July and August. Individuals in the middle quintile of neonatal 25(OH)D_3 had lower odds of sustaining a fracture than did those in the lowest quintile (adjusted OR: 0.75; 95% CI: 0.58, 0.96), but a global test did not show any significant overall association (adjusted $P = 0.13$).

Conclusions: This study suggested that neonatal vitamin D status does not influence subsequent fracture risk in childhood. This is in accordance with studies that report no association between antenatal maternal vitamin D status and childhood fractures. Further studies are needed to examine fracture risk in relation to prenatal vitamin D status in a randomized controlled setting. *Am J Clin Nutr* 2017;106:155–61.

Keywords: fractures, vitamin D, dried blood spots, epidemiology, osteoporosis, development

INTRODUCTION

Globally, the fracture incidence of the forearm, hand, and foot is high among healthy children, but it varies across countries, ages, sexes, and sites (1–5). Multiple factors contribute to the increased risk of pediatric fractures, such as low bone mass; socioeconomic status; childhood obesity and lifestyle, i.e., physical activity (risk-taking behavior); and diet composition (6–8). However, whether antenatal and neonatal vitamin D status has clinical relevance in fracture prevention has not been examined extensively, and only a few studies, to our knowledge, have related maternal vitamin D status to offspring fracture risk and found no association (9, 10). However, in a sensitivity analysis in the Danish Fetal Origins 1988 cohort among 850 mother-offspring pairs, Petersen et al. (9) found a borderline inverse association between 25-hydroxyvitamin D [25(OH)D] as a continuous variable and offspring forearm fractures ($P = 0.054$), but there was no association when 25(OH)D concentrations were categorized according to clinical cutoff values.

On the other hand, observational studies have examined intrauterine vitamin D status (or proxy measures of vitamin D) and long-term bone growth and density among offspring, finding either a direct (11–13) or no association (14). The studies were

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Supplemental Figures 1–4 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Abbreviations used: DBS, dried blood spot; DXA, dual-energy X-ray absorptiometry; ICD-10, International Classification of Diseases, 10th edition; NPR, National Patient Registry; 25(OH)D, 25-hydroxyvitamin D.

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primarily carried out in Caucasian children; however, the studies differed in several respects, such as the study design, the vitamin D assessment method, the geographical latitude of the country of the study population, and age range of the offspring at the outcome measurements.

To our knowledge, no studies have examined neonatal vitamin D status in relation to pediatric fracture risk, yet in Denmark we have optimal opportunities for studying such associations with access to the comprehensive and complete national health registers with long follow-up combined with systematically collected biological material.

Recently, 25(OH)D measured from dried blood spots (DBSs) collected in the context of the American or European newborn-screening programs for phenylketonuria have been used to examine associations between neonatal 25(OH)D concentration and the development of numerous other outcomes, such as schizophrenia (15), childhood brain tumor risk (16), type 1 diabetes (17, 18), multiple sclerosis (19, 20), autism (21), inflammatory bowel disease (22), and cardiovascular disease risk markers (23), but measured neonatal 25(OH)D concentrations from DBSs have not been previously compared among pediatric fracture cases and controls.

We used a case-cohort sampling design to compare $25(OH)D_3$ concentrations in neonatal DBS samples retrieved from the Danish Biological Specimen Bank for Neonatal Screening for individuals who developed fractures and for a random subcohort.

METHODS

Participants

The Danish Civil registration system was used to identify all live-born children during 1981–2002 (n = 1,360,466), and a random subcohort thereof was sampled for 25(OH)D analyses to be used across the D-tect studies (n = 3585) (24). Individuals from twin or multiple births were allowed in the sample. The Danish National Patient Registry (NPR) was used as the source to assess fracture outcome. The NPR is a mandatory nationwide health registry that was established in 1977. There have been important changes over time in the information reported to the NPR. Most importantly for our data, mandatory registration of outpatients and patients from emergency rooms was initiated in 1995 (25), whereas before 1995 it was only mandatory to register hospitalized fracture cases. The result was a substantial increase in the number of fracture cases registered from 1995 onward. To avoid this sudden increase in registered fracture cases, in combination with a prespecified age group of interest (6–13 y), we selected individuals only from the main subcohort who were live-born during 1 January 1989–31 December 1999 in Denmark and had DBS cards with sufficient material for analysis and available vitamin D results (n = 1600). Also, a random sample was selected of 1039 fracture cases from a total of 82,154 fracture cases who had been admitted to the hospital with a fracture of the forearm, wrist, or scaphoid bone [International Classification of Diseases, 10th edition (ICD-10): S52, S62.0]; the clavicle (ICD-10: S42.0); or the ankle (ICD-10: S82.5, S82.6, S82.8), because these sites are the most common among European children (5). For the present study, information on the cause of fracture had not been assessed from the NPR. The NPR includes diagnosis codes and procedure codes for all treatments and contains outpatient and emergency room contacts (26). The fractures occurred when the children were 6–13 y old. If the child had >1 fracture admission, we counted only the first admission. There were 4 fracture cases that were also part of the random subcohort, which we attributed to the random subcohort. The flowchart for the study population is presented in **Figure 1**.

The Danish registers used in this study have coverage of almost the entire population and good validity of fracture diagnosis recordings in the NPR based on adult fracture patients with primary hyperparathyroidism (27). The accuracy is expected to be similar in children, because the treatment of pediatric and adult fractures takes place in the same hospital units.

Ethical considerations

Permission to access and analyze the DBS samples from the Biological Specimen Bank for Neonatal Screening was given by the Danish National Committee on Biomedical Research Ethics (journal no.: H-3-2011-126) and by the steering committee from the biobank. Permission from the Danish Data Protection Agency was also granted (J. no.: 2012–41-116). Anonymous register-based studies are not required ethical approval according to Danish law.

Assessment of vitamin D status

Since 1 May 1981, neonatal DBS samples taken by heel prick ≤1 wk after birth have been collected for all newborns in Denmark. After routine screening for congenital disorders,

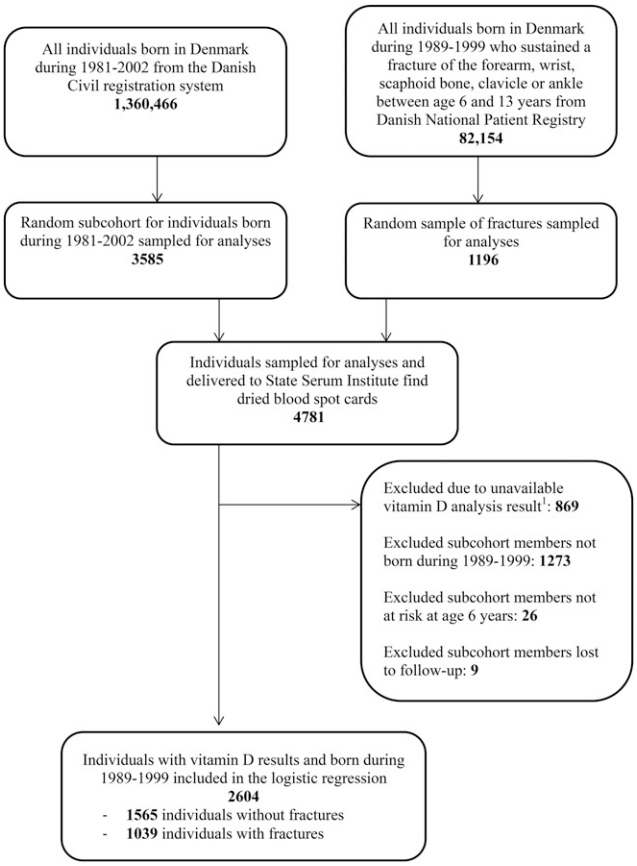

FIGURE 1 Flowchart of the study population. [1]Dried blood spot cards were not found, there was insufficient material for analysis, or the analysis failed.

residual DBS filter cards are stored at −20°C in a locked freezer at the Biological Specimen Bank for Neonatal Screening at the State Serum Institute (28). Previous studies have shown that storage times of $25(OH)D_3$ for >20 y do not seem to bias interindividual variation in concentrations for a given birth cohort regardless of storage temperature and light exposure (29).

From the stored surplus card samples, one 3.2-mm punch, taken halfway from the center of the blood spot was obtained. This punch was used to measure the main circulating form of vitamin D, neonatal $25(OH)D_3$, as well as $25(OH)D_2$ (30), but not the C-3 epimer of $25(OH)D_3$.

The assay used was a highly sensitive liquid-chromatography tandem mass-spectroscopy method run at the State Serum Institute by using a modified version of the method of Eyles et al. (29). There was acceptable precision for all measured concentrations for intra-assay and interassay analyses. The CV for intra-assay and interassay variation for $25(OH)D_3$ ranged 7–12% and 7–20%, respectively. Laboratory investigators were blinded to the diagnosis as well as the season of birth.

Because capillary blood during the neonatal period has significantly higher hematocrit values compared with venous blood (31), we adjusted the concentrations of $25(OH)D_3$ in the DBS samples to equivalent serum concentrations using the following formula: serum $25(OH)D_3$ nmol/L = DBS $25(OH)D_3$ nmol/L × 1/[1 − 0.61 (the hematocrit fraction)] for capillary blood for newborns (31). We omitted all the $25(OH)D_2$ measurements, because only 4.5% were above the detection limit of 3 nmol/L. The concentration of $25(OH)D_3$ is expressed in nmol/L.

Assessments of covariates

Information on some maternal characteristics (educational level, ethnicity) was obtained from Denmark Statistics, and smoking during pregnancy, age, parity, and gestational age were obtained from the Danish National Birth Register. Educational level was divided into 3 levels: *1*) basic school 8th–10th class; *2*) general upper–secondary education, short-cycle higher education or vocational education and training; and *3*) medium- or long-cycle higher education or bachelor. Ethnicity was defined as maternal European or non-European origin. Hence, children with fathers with non-European origin were considered European if mothers were of Western origin. There was information on maternal smoking during pregnancy only for a subsample of a total of 1982 individuals, of which 798 individuals were from the case group, because information on smoking during pregnancy is available from Danish registries for individuals giving birth only from 1991 onward, when the collection of the smoking information started. Since 1997, the data on smoking during pregnancy was collected electronically; thus, the information collected during 1991–1996 may not be complete. The smoking variable from 1991 to 1996 was grouped into smoking and nonsmoking during pregnancy, and from 1997 and onward the cigarette smoking was reported in the following groups: unknown, mother not smoking, mother smokes, mother stopped smoking in first trimester, mother stopped smoking after first trimester, mother smokes up to 5, 6–10, 11–20, or >20 cigarettes/d. When combining these information sources, we categorized the smoking variable into unknown, smoking, and nonsmoking during pregnancy. Mothers were considered smokers if they had ever smoked during pregnancy. The reporting of parity changed during the study period. From 1973 to 1996 the register included information on live and still births only. During this period, we used the sum of births plus the actual births to estimate parity. Subsequently, parity was dichotomized into primiparous and multiparous. Maternal and gestational age were included in the analyses as continuous variables.

Statistical analysis

In the descriptive data, the cases and the individuals from the random subcohort were compared by using the chi-square test for categorical variables and the 2-sample *t* test for continuous variables.

Unlike the classic case-cohort design (32), only a random sample of the fracture cases was included, and because loss to follow-up was rare among the individuals in the subcohort ($n = 9$), the data were analyzed as a case-control sample by including only individuals with complete follow-up.

Using logistic regression, we estimated unadjusted and adjusted ORs and 95% CIs for the risk of fractures of the forearm, wrist, scaphoid bone, clavicle, or ankle, whichever came first, in the ages 6–13 y in relation to $25(OH)D_3$ as a continuous variable or as a categorical variable (quintiles of the distribution of $25(OH)D_3$ in the subcohort) to capture a potential nonlinear relation. Based on our hypothesis that neonatal serum $25(OH)D_3$ concentrations are lower among children who go on to sustain fractures in childhood, the lowest category was chosen as the reference. A priori, the model was adjusted for offspring, sex, gestational age, parity, and maternal educational level, because these factors have been previously associated with neonatal bone mineralization (33). Moreover, we adjusted for maternal age, because maternal age seems to be associated with both vitamin D status during pregnancy as well as offspring fracture risk during childhood (34, 35). Based on theoretical plausibility presented in the background, potential interactions between the vitamin D quintile categories and maternal ethnicity were examined by adding interaction terms to the model and testing the statistical significance.

Because only a limited number of individuals had information on smoking (n = 1982), the likelihood-ratio test was used to estimate whether adjusting for smoking in the model would improve the fit of the logistic model. Also, the individuals with missing information on maternal smoking during pregnancy were compared with the individuals with information on maternal smoking to address selection bias by using the chi-square-test and the 2-sample *t* test. The $25(OH)D_3$ was square-root transformed to achieve normal distribution before performing the 2-sample *t* test. To further explore the role of maternal smoking during pregnancy, sensitivity analyses were performed on the subset with available information on smoking during pregnancy to test if maternal smoking was associated with fracture risk in the offspring by using logistic regression as well as by examining the distribution of maternal smoking during pregnancy in relation to the quintiles of the distribution of $25(OH)D_3$ by using the chi-square test. Also, we tested whether there was effect modification with maternal smoking during pregnancy by adding interaction terms to the model and testing the statistical significance.

Because the 25(OH)D concentrations change according to the seasonal variation in sun exposure at northern latitudes in both pregnant women and their offspring at birth (36), we performed

prespecified sensitivity analysis adjusting for the season of birth and included the season of birth as an interaction term in further analysis. The seasons were defined as November to January, February to April, May to July, and August to October based on the seasonal variation in serum 25(OH)D concentrations among individuals from countries in northern latitudes (36, 37). A likelihood-ratio test was used to estimate whether including ethnicity or the season of birth in the model would improve the fit of the logistic model.

Sensitivity analyses were performed to test whether there was effect modification with sex by adding interaction terms to the model and testing the statistical significance. A likelihood-ratio test was used to estimate whether including sex in the model would improve the fit of the logistic model.

Finally, global tests were performed as sensitivity analysis to examine if there were any overall associations.

Statistical analyses were carried out in Stata version 14.1. A P value of <0.05 was considered significant. Data are expressed as means ± SDs unless otherwise specified.

RESULTS

In the entire sample, the distribution of $25(OH)D_3$ was skewed. For all subjects it was 27.7 ± 18.9 nmol/L [median (IQR): 23.5 nmol/L (13.3, 37.3 nmol/L)] when corrected for the hematocrit fraction in capillary blood. As expected, $25(OH)D_3$ showed statistically significant monthly variation ($P < 0.001$) with the highest values in July and August and the lowest value in April among the individuals from fracture cases and subcohort combined (**Figure 2**).

In **Table 1**, background characteristics are presented for the 1039 fracture cases and the 1600 children from the subcohort. There were no significant differences between the fracture cases and the controls regarding sex, gestational age, birth weight, $25(OH)D_3$ quintile categories, parity, or maternal educational level. There were borderline more maternal smokers among the case group compared with the subcohort (24% compared with 20%, $P = 0.05$), and the individuals with missing information on maternal smoking were born primarily in 1989 and 1990.

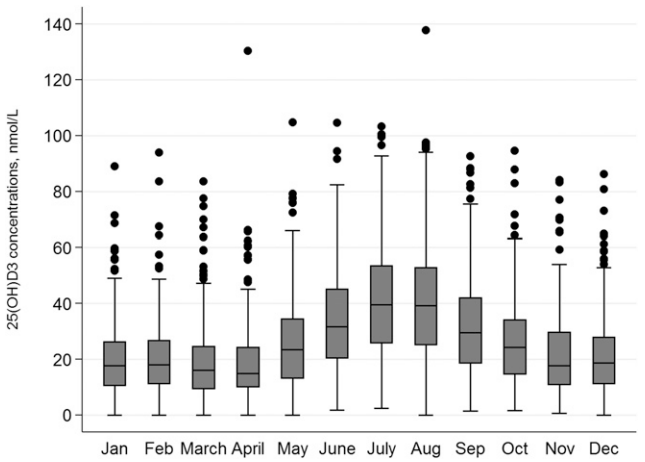

FIGURE 2 Box plot of the median 25(OH)D3 concentrations by the month of birth of the individuals from the total study population ($n = 2604$). The boxes indicate the interquartile range, the vertical lines indicate the range, and the dots indicate outliers. The dried blood spot 25(OH)D3 concentrations have been corrected for the hematocrit fraction of 61% for capillary blood. Test for no interaction between month of birth and sampling status (cases versus subcohort): $P = 0.48$. Test for no difference in 25(OH)D3 concentrations between cases and subcohort adjusted for month of birth: $P = 0.54$. 25(OH)D3, 25-hydroxyvitamin D_3.

Also, there was a lower percentage of individuals with maternal non-European ethnic origin in the case group compared with the subcohort (4.3% in the case group and 7.7% in the subcohort, $P < 0.001$).

The odds for sustaining fractures were lower in the middle (third) quintile of $25(OH)D_3$ in both the crude analysis (OR_3: 0.76; 95% CI: 0.60, 0.98) and the adjusted model 2 (OR_3: 0.75; 95% CI: 0.58, 0.96) (**Table 2**), than in the first quintile. Further adjustment for smoking and season of birth, as well as birth weight, showed a similar pattern as model 2 (Table 2). There was no association between $25(OH)D_3$ as a continuous variable and fracture risk (data not shown). However, the results from the global tests did not show any significant overall associations in any of the models (Table 2).

Maternal smoking during pregnancy as exposure did not predict odds of fractures (OR: 1.19; 95% CI: 0.98, 1.45) but did seem to influence the $25(OH)D_3$ concentrations with the use of $25(OH)D_3$ concentrations both as a continuous variable ($P < 0.0001$) and as quintiles of $25(OH)D_3$ ($P < 0.001$).

There was no significant interaction between categories of vitamin D concentrations and ethnicity, sex, smoking, or season of birth in relation to odds of fractures (**Supplemental Figures 1–4**).

DISCUSSION

In this large, population-based, randomly selected group of children born in Denmark during 1989–1999, we found no evidence of an association between neonatal $25(OH)D_3$ and the odds of sustaining fractures in childhood at age 6–13 y. Moreover, we found no interactions with ethnicity, sex, maternal smoking, or season of birth. In the analysis with maternal smoking during pregnancy as the exposure, the $25(OH)D_3$ concentrations were lower but were not associated with the odds of fracture.

Our findings agree with results from the recent Danish Fetal Origins 1988 Cohort study ($n = 850$) (9) and the Danish National Birth Cohort study ($n = 1497$) (10), showing no association between antenatal 25(OH)D concentration and risk of fractures among the offspring during childhood. Given that the fetus relies entirely on the maternal status (38) and that low bone mineral density is predictive of increased fracture risk (6, 39–45), our results are equivalent to the large observational ALSPAC (Avon Longitudinal Study of Parents and Children) (14) and the randomized controlled MAVIDOS (Maternal Vitamin D Osteoporosis Study) (46) but in contrast to other observational studies that found that prenatal vitamin D status and/or ultraviolet B exposure was associated with skeletal strength and development (11–13).

In the interpretation of the differences in the above-mentioned studies, one may consider the etiology of childhood fractures (47); although the measurements of bone mass possibly capture the underlying skeletal deficits unrelated to the occurrence of an accident, the fracture events may characterize the degree of trauma, which was not distinguished in any of the fracture studies. Unfortunately, the cause of fracture was not retrieved from the Danish National Patient Register for the present study either, but it may be of interest for future studies.

Comparable to previous studies (48–51), our sensitivity analyses suggested that in utero exposure to smoking influenced vitamin D concentrations but not fracture risk. These results make

TABLE 1
Maternal and offspring background characteristics for individuals from the case group and the random subcohort[1]

	Fracture cases		Random subcohort		
	N	Value	*n*	Value	*P*
Offspring					
Fracture event of forearm, wrist, scaphoid bone, clavicle, or ankle at age 6–13 y	1039	100	1565	12.9	<0.001
Girls	1039	50.1	1565	47.7	0.23
Gestational age, wk	1023	39.5 ± 1.8	1537	39.6 ± 2.0	0.28
Birth weight, g	1029	3494 ± 561	1538	3497 ± 578	0.87
Mean $25(OH)D_3$ quintiles (nmol/L)	1039		1565		0.29
1 (7.88)		22.1		19.9	
2 (15.33)		21.1		19.9	
3 (23.90)		17.1		19.9	
4 (33.93)		20.7		20.2	
5 (53.18)		19.0		20.1	
Maternal					
Smoking during pregnancy	1039		1565		0.05
Yes	248	23.9	311	19.9	
No	550	52.9	873	55.8	
Age, y	1036	28.2 ± 4.6	1549	28.5 ± 4.7	0.13
Multiparous, yes	1036	54.5	1548	55.7	0.57
Educational level	1014		1513		0.46
Basic school 8th–10th class		29.6		27.3	
General upper–secondary education, short-cycle higher education, or vocational education and training		49.7		51.4	
Medium or long-cycle higher education or bachelor		20.7		21.4	
Immigrant background	1039		1560		0.001
European		95.7		92.3	
Non-European		4.3		7.7	

[1] Values are percentages or means ± SDs. The cases and the individuals from the subcohort were compared by using the chi-square test for categorical variables and the 2-sample *t* test for continuous variables. $25(OH)D_3$, 25-hyrdroxyvitamin D_3.

us question whether we should adjust for maternal smoking during pregnancy from a confounding perspective. Nevertheless, the inclusion of smoking in model 3a did not improve the fit of the logistic regression, and the pattern of the estimates in this analysis was essentially similar to those from the other models.

The numerical values of $25(OH)D_3$ in the present study were low [$25(OH)D_3$: 27.4 ± 18.5 nmol/L) compared with most other studies using DBS samples (varied from 28.2 to 48.5 nmol/L) (16, 17, 19, 21, 23) but, importantly, also compared with studies measuring $25(OH)D_3$ in a similar subsample of Danish neonates [McGrath et al. (15): 35.9 ± 21.0 nmol/L; Nielsen et al. (20): 33.0 ± 16.9 nmol/L for cases and 35.9 ± 17.5 nmol/L for controls]. A number of potential explanations for the low values may be considered in the interpretation of our findings. Intervariability between laboratories both within and between countries is to be expected (52), especially because there are currently no quality-assurance programs for vitamin D in DBSs. However, our laboratory participates in the Vitamin D External Quality Assessment Scheme with the equivalent-serum method. Dissimilarities in the separation methods may be considered especially because the C3-epiform of $25(OH)D_3$ was not included in our measurements. This is relevant because the liquid-chromatography tandem

TABLE 2
ORs (95% CIs) of fractures in offspring at age 6–13 y for the concentration of $25(OH)D_3$ by the quintiles of the distribution of $25(OH)D_3$[1]

	Model 1 (*n* = 2604)	Model 2 (*n* = 2559)	Model 3a (*n* = 2559)	Model 3b (*n* = 2559)	Model 3c (*n* = 2547)
Mean $25(OH)D_3$ quintiles (nmol/L)					
1 (7.88)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
2 (15.33)	1.03 (0.81, 1.31)	1.00 (0.78, 1.28)	1.01 (0.79, 1.30)	0.98 (0.76, 1.25)	0.99 (0.77, 1.26)
3 (23.90)	0.76 (0.60, 0.98)	0.75 (0.58, 0.96)	0.76 (0.59, 0.98)	0.72 (0.55, 0.93)	0.74 (0.57, 0.95)
4 (33.93)	0.98 (0.77, 1.24)	0.94 (0.73, 1.20)	0.96 (0.74, 1.23)	0.88 (0.68, 1.14)	0.92 (0.72, 1.18)
5 (53.18)	0.92 (0.72, 1.17)	0.89 (0.69, 1.15)	0.91 (0.70, 1.17)	0.81 (0.62, 1.07)	0.88 (0.68, 1.14)
Global test, *P*	0.13	0.13	0.16	0.08	0.14

[1] Model 1: logistic regression. Model 2: model 1 combined with adjustment for sex, gestational age, parity, maternal educational level, maternal ethnicity, and maternal age. Model 3a: model 2 combined with adjustment for maternal smoking during pregnancy; likelihood-ratio test: $P = 0.21$. Model 3b: model 2 combined with adjustment for the season of birth; likelihood-ratio test: $P = 0.43$. Model 3c: model 2 combined with adjustment for birth weight; likelihood-ratio test: $P = 0.07$. $25(OH)D_3$, 25-hyrdroxyvitamin D_3; ref, reference.

mass-spectroscopy assays have shown elevated concentrations of $25(OH)D_3$ in infants because of the presence of the C-3 epimer of $25(OH)D_3$ (53). Additional explanations may be related to deviation from detailed guidelines of the blood collection procedure, where the most common errors are overfillings, partial fillings, or multiple applications on the circles on the filter paper (54); punch position, because findings have revealed that 25(OH)D concentrations differ across the spots, with the highest concentrations in the periphery of the spots and lowest in the center (54); and/or multiple freeze-thawing cycles, as a result of the stored DBS samples from the 1980s being subjected to many punches in relation to analysis in other previous research projects. Multiple freeze-thaw cycles in serum 25(OH)D seem to be valid (55), and we assume that the validity is also applicable to 25(OH)D measurements in DBS samples, although this has not been formally tested.

However, we have no reason to believe that the generally low values influenced the ranking of subjects and hence no reason to question the validity of our associations. Indeed, the seasonal variation in the $25(OH)D_3$ concentrations was well captured in the present study for both the cases and the subcohort.

Strengths and limitations of the study

There are some strengths and limitations to the study that need to be taken into account in the interpretation of the findings (56). The strengths of the present study lie in the large sample size of individuals randomly selected from the entire Danish population coupled with the neonatal biomaterial ($n = 2639$). All but one (14) of the previously published studies that we are aware of included <1500 mother-offspring pairs. Also, apprehension regarding selection bias was negligible because information on the mother-offspring pairs in the present analyses was derived from national registries, and the obtainment of the Danish neonatal DBS samples are close to complete. Even though we were able to adjust for several potential confounders through the use of information from comprehensive registers with high validity and covering the entire Danish population, there may still be a risk of residual confounding from variables for which we lacked information, although we adjusted for sociodemographic factors, such as educational level and maternal age, as well as behavioral factors, such as parity and maternal smoking, which may be considered proxy variables for other life-style factors in general, including supplement use and physical activity level (56, 57).

Implication for policy makers

Policy-making strategies in relation to primary prevention of fractures among children cannot be derived from the results at this point, but ongoing, randomized controlled trials with supplementation of vitamin D in pregnancy (46, 58, 59) await the first bone-fracture outcome data in the children to properly inform health policy.

Conclusion and future studies

Overall, neonatal vitamin D status does not seem to influence the subsequent fracture risk in childhood. These results are in line with results from the few previous studies that examined associations between antenatal maternal vitamin D status and childhood fractures. Large, randomized controlled trials with vitamin D supplementation during pregnancy in relation to fracture outcome are needed at this point.

The authors' responsibilities were as follows—MNH, BA, and BLH: initiated the study and participated in its design and coordination; MNH and PF: performed the statistical analysis; AC: performed the 25(OH)D measurements on the DBS samples; MNH, BA, and BLH: wrote the manuscript with contributions from all authors; MNH: had primary responsibility for the final content and affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained; and all authors: were responsible for interpreting the data and read and approved the final manuscript. CC has received consultancy, lecture fees, and honoraria from Alliance for Better Bone Health, Amgen, Eli Lilly, GSK, Medtronic, Merck, Novartis, Pfizer, Roche, Servier, Takeda, and UCB. BA conducts epidemiological studies through research contracts between his institution and Novartis and UCB Pharma. None of the other authors reported a conflict of interest related to the study.

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